Environmental Mutagens & Carcinogens 23-1 : 35-40 (2003)

Bioassays of Polycyclic Aromatic Hydrocarbons in ZR-75-1 Human Breast Cancer Cells

Kyung N. Min and Yhun Y. Sheen*

College of Pharmacy, Ewha Womans University, # 11-1, Daehyun-dong, Sudaemun-ku, Seoul, 120-750, Korea

(Received February 2, 2003 / Accepted February 24, 2003)

ABSTRACT : **Polycyclic aromatic hydrocarbons (PAH) are frequently detected in food, water, soil, and sediment and are widespread environmental pollutants formed by the incomplete combustion of fossil fuels, woods and other organic matter. PAHs are considered to be probable human carcinogens. The mechanism of action of PAHs has been studied extensively, however it is not clear how PAHs turn on CYP1A1 in human breast cancer. Our laboratory have been studied the effect of PAHs in the human breast cancer cell MCF7. In this study, we examined the ZR-75-1 human breast cancer cells as a new system to evaluate bioactivity of PAHs. ZR-75-1 human breast cancer cell line responses to estrogen and progesteron. We have been able to estbilish long term culture system of this cells then used for the study to observe the effect of PAHs. We demonstrate that PAHs induced the CYP1A1 promoter and 7-ethoxyresolufin O-deethylase (EROD) activity in a concentration-dependant manner. RT-PCR analysis indicated that PAHs significantly up-regulate the level of CYP1A1 mRNA. Some of PAHs showed stronger stimulatory effect on CYP1 gene expression than TCDD. Apparently, ZR-75-1 cells have Aryl hydrocarbon receptors, therefore it would be good experimental tool to study the cross-talk between PAHs and steroid actions.**

Key words : **PAH, EROD, TCDD, ZR-75-1**

Introduction

Recent industrial society has human widely exposed to PAHs (polynuclear aromatic hydrocarbons) that are comming from the incomplete combustion of organic material as wider spread environmental contaminants (Menzie *et al.*, 1992). Biological activities of PAHs are not known although PAHs are considered as carcinogens. PAHs such as benzo(a)pyrene (B[a]P) undergo metabolic activation by cytochrome P450 and epoxide hydrolase to chemically reactive ultimate carcinogen diol epoxides (Chou *et al.*, 1986). Mutagenic and carcinogenic potency of many PAH metabolites has been demonstrated in vivo rodent assays and in vitro short-term assays (Hecht *et al.*, 1994; Zaho and Ramos, 1998). The mechanisms of PAH bioactivation was also studied in human cDNA expressed CYP1A1 and purified CYP1A1, and results showed the enhancement of the genotoxicity of a proximate carcinogenic form of benzo[a]pyrene (Shimada *et al.*, 1994). cDNA expressed CYP1A1 and CYP1A2 both catalyzed stereoselective epoxidation of a series of PAHs (Shou *et al.*, 1996). Human CYP1B1 has also recently been demonstrated to be capable of bioactivating PAH carcinogens (Shimada *et al.*, 1996; Luchi *et al.*, 1999). PAHs such as TCDD induce the expression of *CYP1* family, which consists of at least three enzymes, *CYP1A1*, *CYP1A2* and *CYP1B1* has been shown to be important in the metabolism of several xenobiotics such as PAH and heterocyclic amines. TCDD induction of *CYP1* transcription is mediated by the cytosolic AhR, which is known as a ligand-activated transcription factor. The activation of AhR involves ligand binding, dissociation of heat-shock protein-90, nuclear translocation, and dimerization with the Arnt followed by binding to dioxin responsive element (DRE, or XRE) enhancer elements in the 5'-noncoding region of the responsive gene (Carrier *et al.*, 1992; Swanson *et al.*, 1993; Denison and Whitlock, 1995). The mechanism of action of this compound is to activate the AhR to a form that binds to specific gene regulatory sequence elements, called XREs, through heterodimerization with Arnt (Dolwick *et al.*, 1993; Mason *et al.*, 1994; Ko *et al.*, 1996). AhR and Arnt have a similar overall structure and belong to the basic helix-loop-helix class of transcription factors (Pendurthi *et al.*, 1993; Poland *et al.*, 1994). Members

*To whom all correspondence should be addressed

in this class of factors are characterized by a bHLH motif contiguous with a region, PAS (Per-Arnt-Sim), which is conserved between the *Drosophila* neural cell developmental regulator Sim, the *Drosophila* circadian rhythm regulatory protein, Per, and Arnt (Littlewood and Evan, 1995). Upon binding XREs, the AhR-Arnt complex activates transcription of adjacent structural gene encode enzymes that are involved in the oxidative metabolism of these compounds (Whitelaw *et al.*, 1994; Whitlock *et al.*, 1996). In this study, we tried to develop the bioassay system of PAHs based on their ability to induce CYP1A1. We measure the reporter gene activity as a AhR activation in mouse liver ZR-75-1 cells transfected with pCYP1A1-Luc to monitor the induction of cytochrome P4501A1 activity with PAHs treatment. Also we measured the increase in 7-ethoxyresorufin-O-dethylase (EROD) production as an indication of induction of CYP1A1 catalytic activity by PAHs treatments activation in ZR-75-1 human breast cancer cells.

Materials and Methods

Materials

2,3,7,8-tetrachloro-r-dioxin was kindly provided by Dr. K. Chae from NIEHS, (Research Triangle Park, NC, U.S.A.). Agarose was purchased from FMC, Hind III were ordered from by Gibco BRL. Tfx^{TM}-50, pGL3 basic vector and luciferase assay system were purchased from Promega.

Construction of *Cyp1a1*-Luc

Human CYP1A1 5'-flanking DNA was cloned into pGL3 vector at *Hin*dIII site.

Cell culture and transfection

ZR-75-1 human breast cancer cells were cultured in RPMI 1640 media supplemented with 10% (v/v) heat inactivated - fetal bovine serum and penicillin-streptomycin (100 U/ml). For the transfection of phCYP1A1-luc, ZR-75-1 cells were seeded in 48-well plates. 150 ng of phCyp1A1-luc and 0.63 μl of Tfx^{TM}-50 (Promega) were mixed in 100 μl of serum-free medium and incubated at room temperature for 15 minutes before being added to each well. Cells were incubated for at least 1 hr before cells were maintained in normal RPMI 1640 media containing 10% heat inactivated - fetal bovine serum. The details were followed as supplier's manual.

Chemical treatment

ZR-75-1 cells were rinsed with PBS three-times before the administration of various chemicals in serum-free medium. Stock solutions of chemicals were made in dimethyl sulfoxide (DMSO) as a vehicle and control cells were treated with 0.1% DMSO. Either 1 nM TCDD or PAHs (all 106, 107, 108 M) was administered for 24 hr. Final concentration of DMSO didnt exceed 0.1%.

Luciferase reporter assay

Luciferase assays were performed using the Luciferase Assay System (Promega). Briefly, the transfected cells were lysed with reporter lysis buffer. The lysates was incubated with luciferase substrate, and luciferase activity was determined with a luminometer. Protein assay of cell extracts was carried out using the Micro BCA protein assay reagent kit (Pierce) and an ELISA Reader (Bio-rad). Luciferase activity was normalized to protein content. The data are presented as the fold induction of control cells that were treated with 0.1% DMSO.

EROD assay

Ethoxyresorufin-0-dealkylase (EROD) activity was basically assayed as described by Kennedy S.W. with some modification. Cells were seeded in 48 well plates, allowed to attain 60% confluency, and adapted with serum free media for 24 hr. Cells were treated with TCDD and PAHs at the indicated concentration in legend. Sodium phosphate buffer was added to each well of 48 well plate. And then cells were pre-incubated with ethoxyresorufin (Sigma) at 37°C for 10 min. The reaction was started by adding β-nicotinamide adenine dinucleotide phosphate reduced form (β-NADPH, Sigma). After incubation for 15 min in 37°C, the reaction was stopped by adding acetonitrile containing fluorescamine (Sigma). After 15 min the plates were scanned for resorufin (Sigma) with a 530 nm excitation and a 590 nm emission, and for proetin with a 360 nm excitation and a 460 nm emission. Fluorescence data were imported into Table Curve (Jandel scientific) for curve fitting.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNAs were exrtacted using Trizol reagent (Gibco-BRL) according to the manufacturer's

instructions and cDNA were synthesized from 3 μg total RNA using reverse transcriptase (Gibco-BRL) in the presence of random primers in a 20 μl reaction volume at 37°C for 60 min.

1 μl of cDNA solution was amplified by Taq polymerase (Promega) in a volume of 10 μl. For mRNA detection of β-actin, GAPDH, mouse cyp1a1 and human CYP1A1 genes the PCR procedure was performed with 25 cycles of denaturation at 94°C for 0.5 min, annealing at 62°C for 0.5 min, and extention at 72°C for 1 min, with a pre-denaturing time of 5 min and a final extension time of 7 min. The primer sequences were GAPDH (150 bp), 5'-ACATCGCTCAGACACCATGG-3' (sense) and 3'-GGGAAGTAACTGGAGTTGATG-5' (antisense); human CYP1A1 (146 bp), 5'-TAGACACTGATCTG GCT GCAG-3' (sense) and 3'-GGGAAGGCTCCATCAGC-ATC-5' (antisense).

Following PCR amplification, the PCR products were run on a 2% agarose gel with ethidium bromide. The total band volumes of amplified products were calculated by alpha-image analyzer. All glassware and plasti c ware was treated with diethyl pyrocarbonate (DEPC) and autoclaved.

Results

Effects of 13 PAHs on the human *CYP1A1* promoter activity

1 uM of 13 different PAHs and 1 nM TCDD were administered into ZR-75-1 cells transfected with expression plasmid that has human *CYP1A1* 5'flanking DNA 1.6 Kbs cloned in front of luciferase gene for 24 hrs, and the *CYP1A1* promoter activity was monitored by measuring luciferase activity. As shown in Fig. 1, 1 uM benzo(k)fluoranthene showed 100-fold induction of luciferase activity over control cells, and 1nM TCDD showed 38-fold induction of *CYP1A1* promoter activity over control cells. 1 uM dibenzo(a,h)anthracene treatment showed 50-fold induction, 1 uM benz(a)anthracene treatment showed 8-fold induction, 1 uM chrysene treatment showed 14-fold induction over control, respectively. Among 13 different PAHs tested, 1 uM fluoranthene treatment or 1 uM benzo(b)fluoranthene treatment or 1 uM Carbazole treatment or 1 uM phenanthrene or 1 uM acenaphthene treatment or 1 uM anthracene treatment or 1 uM naphthalene or 1 uM pyrene or 1 uM phenanthrene treatment or 1 uM fluoranthene treatment did not induce luciferase activity

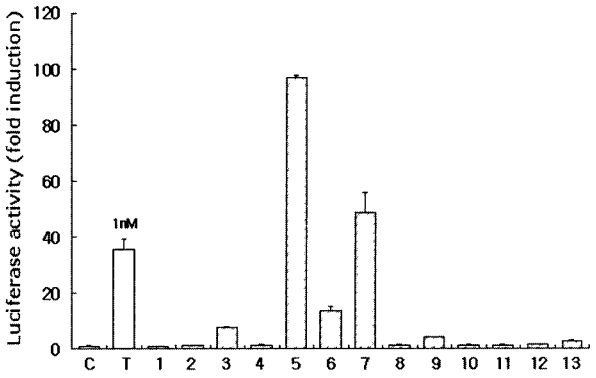

Fig. 1. The effect of PAHs on the luciferase activity in ZR-75-1 cells transfected with *phCYP1A15'-Luc*. After transfection, cells were treated with 1 nM TCDD or 1 μM PAHs for 24 hours. The amount of luciferase transcription was normalized to the amount of protein. The data represent mean±S.D. (n=3) C: Control, T: 2,3,7,8-tetrachlorodibenzodioxin, 1: Acenaphthene, 2: Anthracene, 3: Benz(a)anthracene, 4: Benzo(b)fluoranthene, 5: Benzo(k)fluoranthene, 6: Chrysene, 7: Dibenzo(a,h)anthracene, 8: Fluorene, 9: Fluoranthene, 10: Naphthalene, 11: Pyrene, 12: Phenanthrene, 13: Carbazole.

over untreated control ZR-75-1 cells (Fig. 1). The biological activities of selected 13 PAHs in terms of *CYP1A1* promoter activity stimulation varied considerably. Fluoranthene or acenaphthene or anthracene or fluorene or naphthalene or pyrene or phenanthrene or carbazole showed no response. Benz(a)anthracene, or chrysene showed weak response. Dibenzo(a,h)anthracene or

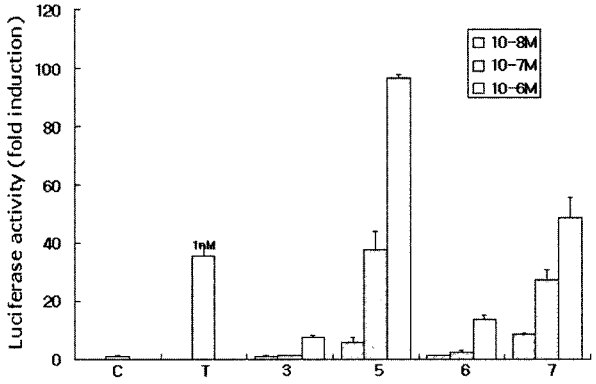

Fig. 2. The dose effect of PAHs on the luciferase activity in ZR-75-1 cells transfected with *phCYP1A15'-Luc*. After transfection, cells were treated with 1 nM TCDD, or 1 μM, 0.1 μM, 0.01 μM PAHs for 24 hours. The amount of luciferase transcription was normalized to the amount of protein. The data represent mean± S.D. (n=3) C: Control, T: 2,3,7,8-tetrachlorodibenzodioxin, 3: Benz(a)anthracene, 5: Benzo(k)fluoranthene, 6: Chrysene, 7: Dibenzo(a,h)anthracene.

benzo(k)fluoranthene showed strong response to *CYP1A1* promoter activity stimulation.

Three different concentrations (0.01 uM, 0.1 uM, 1 uM) of four responding PAHs, such as benz(a)anthracene, benzo(k)fluoranthene, chrysene, and dibenzo(a, h)anthracene in their potency to induce *CYP1A1* promoter activity, were measured in the ZR-75-1 cells transfected with *CYP1A1*-Luc expression plasmid. All four tested PAHs showed concentration dependent stimulation of luciferase reporter gene expression (Fig. 2). These results suggested that four responding PAHs, such as benz(a)anthracene, benzo(k)fluoranthene, chrysene, and dibenzo(a, h)anthracene might be mediated through arylhydrocarbon receptor system.

Effects of 13 PAHs on the EROD activity in ZR-75-1

The effects of 13 selected PAHs on EROD induction were studied in the ZR-75-1 human breast cancer cell bioassay system. 13 selected PAHs and TCDD as a reference were measured in their capability to stimulate EROD activity at 1 uM concentration for PAHs and 1 nM for TCDD. As the results shown in Fig. 3, acenaphthene, anthracene, fluorene, fluoranthene, naphthalene, pyrene, phenanthrene, and carbazole showed no or a very weak response on EROD activity in human breast cancer cells. Benzo(k)fluoranthene, chrysene, and dibenzo(a,h)anthracene showed strong response to stimulate EROD activity in human breast cancer cells (Fig. 3). EROD inducing activity of three different concentrations (0.01 uM, 0.1 uM, 1 uM) of four responding PAHs such as, benz(a)anthracene, benzo(k)fluoranthene, chrysene, and dibenzo(a,h)anthracene were measured in ZR-75-1 cells *in vitro* bioassay. As shown in Fig. 4, benz(k)fluoranthene, and dibenzo(a,h)anthracene resulted in concentration dependent response to EROD induction. Benzo(k)fluoranthene, and dibenzo(a,h)anthracene responded strongly to EROD and *CYP1A1* promoter activity. Benz(a)anthracene and chrysene seemed to respond less to EROD than *CYP1A1* promoter activity.

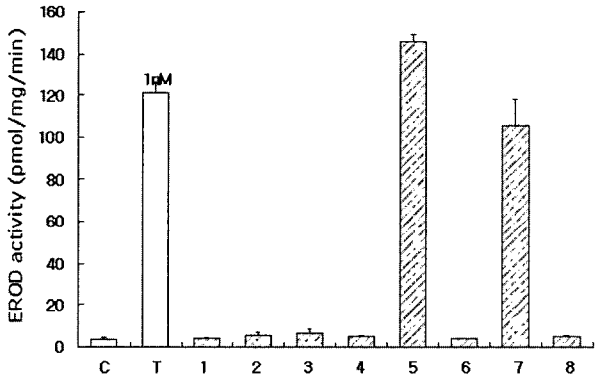

Fig. 3. The effect of PAHs on the EROD activity in ZR-75-1 cells. Cells were treated with 1 nM TCDD or 1 μM PAHs for 24 hours. EROD activity was determined as described in methods. The data represent mean±S.D. (n=3) C: Control, T: 2,3,7,8-tetrachlorodibenzodioxin, 1: Acenaphthene, 2: Anthracene, 3: Benz(a)anthracene, 4: Benzo(b)fluoranthene, 5: Benzo(k)fluoranthene, 6: Chrysene, 7: Dibenzo(a,h)anthracene, 8: Fluorene, 9: Fluoranthene, 10: Naphthalene, 11: Pyrene, 12: Phenanthrene, 13: Carbazole.

Effect of 13 PAHs on the level of CYP1A1 mRNA

1 μM of different PAHs and 1 nM TCDD were administered into human breast cancer ZR-75-1 cells for 6 hours and the level of CYP1A1 mRNA were measured by RTPCR as described in methods.

As shown in Fig. 5, 1 μM benz(a)anthracene treatment showed 9-fold induction of CYP1A1 mRNA level, 1 μM benzo(k)fluoranthene treatment showed 12-fold induction, 1 μM chrysene treatment showed 8-fold induction, 1 μM dibenzo(a,h)anthracene treatment showed 11-fold induction. 1 μM benzo(b)fluoranthene treatment showed 5-fold, 1 μM fluoranthene treatment showed 4-fold, 1 μM naphthalene treatment showed 4-fold of CYP1A1 mRNA level over control, respectively. Among 13 different PAHs tested, 1 μM acenaphthene or 1 μM anthacene or 1 μM fluorene,or 1 μM naphthalene did

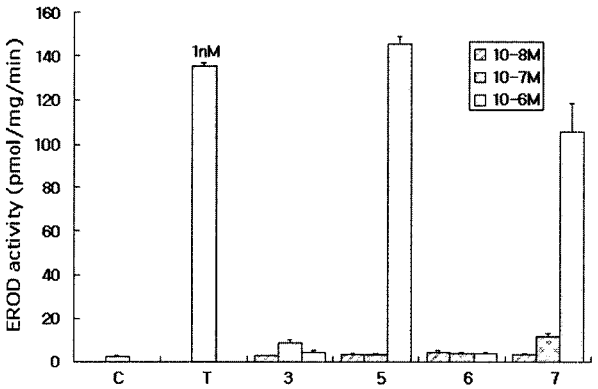

Fig. 4. The dose effect of PAHs on the EROD activity in ZR-75-1 cells. Cells were treated with 1 nM TCDD, or 1 μM, 0.1 μM, 0.01 μM PAHs for 24 hours. EROD activity was determined as described in methods. The data represent mean±S.D. (n=3) C: Control, T: 2,3,7,8-tetrachlorodibenzodioxin, 3: Benz(a)anthracene, 5: Benzo(k)fluoranthene, 6: Chrysene, 7: Dibenzo(a,h)anthracene.

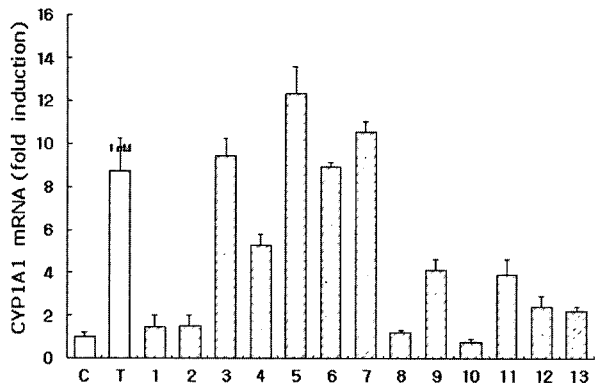

Fig. 5. The Effect PAHs on CYP1A1 mRNA in ZR-75-1 cells. Cells were treated with 1 nM TCDD or 1 μM PAHs for 6 hours and analysed by RT-PCR as described in methods. Each RT-PCR product was analysed by image analyser. The amount of CYP1A1 mRNA was normalized to the amount of GAPDH mRNA. The data represent mean±S.D. (n=3) C: Control, T: 2,3,7,8-tetrachlorodibenzodioxin, 1: Acenaphthene, 2: Anthracene, 3: Benz(a)anthracene, 4: Benzo(b)fluoranthene, 5: Benzo(k)fluoranthene, 6: Chrysene, 7: Dibenzo(a,h)anthracene, 8: Fluorene, 9: Fluoranthene, 10: Naphthalene, 11: Pyrene, 12: Phenanthrene, 13: Carbazole.

not increase CYP1A1 mRNA level over untreated control cell, and 1 μM phenanthrene or 1 μM carbazole treatment showed weak stimulatory effect on CYP1A1 mRNA level over control.

Discussion

Our data from this study showed that ZR-75-1 human breast cancer cells responded to PAH as other hepatic cell line did in terms of *CYP1A1* promoter activity, EROD and CYP1A1 mRNA level increases. In other words, ZR-75-1 cells could be used for the bioassay of PAHs, although some PAHs were responded differently between breast cancer cell line and hepatic cell lines. Benz(a)anthracene, benzo(b)fluoranthene, chrysene were strong stimulators of *CYP1A1* promoter and EROD stimulators in mouse heap 1 cells, however, these PAHs showed very weak activity in ZR-75-1 human breast cancer cells in term of stimulation of CYP1A1 promoter activity and EROD. 1 μM benz(a)anthracene or 1 μM chrysene showed very strong stimulation of CYP1A1 mRNA level which was compatible to 1 nM TCDD effect (Fig. 5), although the CYP1A1 mRNA increase was not correlated to the EROD and CYP1A1 promoter activity. At the moment, we did not understand, however some PAHs such as benzo(k)fluoranthene, dibenzo(a,h)anthracene did stimulated CYP1A1 promoter activity, EROD, and CYP1A1 mRNA level some PAHs such as benzo(a)anthracene, chrysene stimulated only CYP1A1 mRNA level. This study showed that the inducibility of CYP1A1 promoter activity by TCDD was correlated to that of EROD in ZR-75-1 cells. As shown in our study, PAHs with three aromatic rings are hardly capable to induce EROD activity in ZR-75-1 cells. The results suggest that PAH with three rings structure may not meet the structural requirements to bind to the Ah-receptor. Piskorska-Pliszczynska *et al.* (1986) also suggested that low receptor binding affinities are of major importance to the non-responsiveness as generally observed for these compounds. When our results were compared to the results obtained in other test systems including rat hepatocyte (Till *et al.*, 1999), a rat hepatoma cell line (Willett *et al.*, 1997), rainbow trout liver cell line (Bols *et al.*, 1999), a fish cell line (Fent *et al.*, 2000) and the CALUX assay (Machala *et al.*, 2001), it was shown that anthracene, naphthalene, and phenanthrene did not show an effect in any of the difference test system and consequently could be regarded as non-responders (Willett *et al.*, 1997; Bola *et al.*, 1999, Till *et al.*, 1999; Fent *et al.*, 2000). In our experiment, benzanthracene and chrysene showed weak response based on CALUX assay in ZR-75-1 cells as well as EROD bioassay in ZR-75-1 (Figs. 2 and 4). Our data showed that both EROD bioassay and CALUX assay resulted in the same strong response with benzo(k)fluoranthene, and dibenzo(a,h)anthracene. However, with weak responding PAHs based on CALUX assay such as benzanthracene and chrysene showed no response by EROD bioassay in ZR-75-1 cells. This strongly suggested that CALUX assay might give more sensitive measurement of PAHs that EROD bioassay. This might indicated that CALUX assay might be suitable for high throughput screening bioassay system.

Acknowledgment

This research was supported by the grant #R06-2002-011-50 from the KOSEF of Korea.

References

Carrier, F., Chang, C., Duh, J., Nebert, D.W. and Puga, A. (1994): Interaction of the regulatory doma ins of the murine cyplal gene with two DNA-binding proteins in addition to the Ah receptor and the Ah receptor nuclear

translocator (ARNT). *Biochem. Pharmcol.*, **48**, 1767-1778.

Chou, M.W., Helich, R.H. and Fu, P. (1986): Methabolism of 1-nitrobenzo-(a)pyrene by rat liver microsomes to potent mutabolites. *Carcinogenesis*, **7**, 1837-1844.

Denison, M.S. and Whitlock, J.P. (1985): Xenobiotic-inducible transcription of cytochrome P450 genes (Minireview). *J. Biol. Chem.*, **270**, 18175-18178.

Dolwick, K.M., Swansor, H.I. and Bradfield, C.A. (1993): Cloning and expression of a human Ah receptor cDNA. *Mol. Pharm.*, **44**, 911-917.

Piskorska-Pliszczynska, J., Keys, B., Safe, S. and Newman, M.S. (1986): The cytosolic receptor binding affinities and AHH induction potencies of 29 polynuclear aromatic hydrocarbons. *Toxicology Letters*, **34**, 67-74.

Willett, K.L., Gardinali, P.R., Sericano, J.L., Wade, T.L. and Safe, S.H. (1997): Characterization of the H4IIE rat hepatoma cell bioassay for evaluation of environmental samples containing polynuclear aromatic hydrocarbons (PAHs). *Archives of Environmental Contamination and Toxicology*, **32**, 442-448.

Ko, H.P., Okino, S.T., Ma, Q. and Whitlock, J.P. Jr. (1996): Dioxin-induced *Cyp1a1* transcription *in vivo*: The aromatic hydrocarbon receptor mediates transactivation, enhancer-promoter communication, and changes in chromatin structure. *Mol. Cell Biol.*, **16**, 430-436.

Littlewood, T.D. and Evan, G.I. (1995): Transcription factors 2: helix-loop-helix. *Protein Profile*, **2**, 621-702.

Mason, G.F., Witte, A.-M., Whitelaw, M.L., Antosson, C., McGuire, J., Wilhelmsson, A., Poellinger, L. and Gustafsson, J.-A. (1994): Purification of the DNA binding form of dioxin receptor. *J. Biol. Chem.*, **269**, 4438-4449.

Machala, M., Matlova, L., Svoboda, I. and Nezvada, K. (1996): Induction effects of polychlorinated biphenyls, polycyclic aromatic hydrocarbons and other widespread aromatic environmental pollutants on microsomal monooxygenase activities in chick embryo liver. *Archives of Toxicology*, **70**, 362-367.

Till, M., Riebniger, D., Schmitz, H.J. and Schrenk, D. (1999): Potency of various polycyclic aromatic hydrocarbons as inducers of CYP1A1 in rat hepatocyte cultures. *Chemico-Biological Interactions*, **117**, 135-150.

Bols, N.C., Schirmer, K., Joyce, E.M., Dixon, D.G., Greenberg, B.M. and Whyte, J.J. (1999): Ability of polycyclic aromatic compounds to induce 7-ethoxyresorufin-O-deethylase activity in a trout liver cell line. *Ecotoxicology and Environmental Safety*, **44**, 118-128.

Pendurthi, U.R., Okino, S.R. and Tukey, R.H. (1993): Accumulation of the nuclear dioxin (Ah) receptor and transcriptional activation of the mouse Cyp1a-1 and Cyp1a-2 genes. *Arch Biochem Biophys*, **306**, 65-69.

Poland, A., Palen, D. and Glover, E. (1994): Analysis of the four alleles of the murine arylhydrocarbon receptor. *Mol. Pharm.*, **46**, 915-921.

Swanson, H.I., Chan, W.K. and Bradfield, C.A. (1995): DNA binding specificites and pairing rules of the Ah receptor, ARNT, and SIM proteins. *J. Biol. Chem.*, **270**, 26292-26302.

Whitlock, J.P., Okino, S.T., Dong, L., Ko, H.P., Clarke-Katzenberg, R., Ma, Q. and Li, H. (1996): Induction of cytochrome P4501A1: A model for analyzing mammalian gene transcription. *FASEB J.*, **10**, 809-817.

Whitelaw, M.L., Gustafsson, J.A. and Poellinger, L. (1994): Identification of transactivation and repression functions of the dioxin receptor and its basic helix-loop-helix/PAS partner factor ARNT. *Mol. Cell Biol.*, **14**, 8343-8355.